Phytophthora ramorum
APHIS Response Protocol
For Forest and Wildland Environments

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(Host List Updated 11 September 2006)

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Response Protocol for Forest and Wildland Environments (outside the infested areas) for infestations of *Phytophthora ramorum*

This document describes the notification procedures required by The United States Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) if *Phytophthora ramorum* is found in a forest or wildland environment. Decisions regarding regulatory action choices within a state are the purview of the State Regulatory Official. This document outlines protocols for eradication and suppression alternatives. Eradication programs can minimize the size of the quarantine area. Suppression projects, which only reduce the rate of spread of the disease can result in a larger quarantine area. Failure of a state to take regulatory action can be expected to result in the entire state being quarantined by USDA, APHIS. Appendices provide examples of survey, sampling and treatment protocols.

1. BACKGROUND, NEED AND OBJECTIVES

Phytophthora ramorum poses a potential threat to forest ecosystems of the United States. Spread of the pathogen has occurred through many means. These include:

- Movement of nursery stock that has inadvertently been shipped, and planted into the environment.
- Natural spread through air, soil, water or wildlife.
- Artificial spread via wood, soil, greenery, green waste or other means.

(See http://www.suddenoakdeath.org for details on disease impacts.)

The means of pathogen spread in wildlands, and associated environmental and climatic conditions conducive for disease establishment and intensification are poorly understood. Recent detections on beech and red oaks in Europe increase the concern that *P. ramorum* may be able to infect forest trees in the Eastern and Midwestern U.S. as these events provide evidence of susceptibility, in natural settings, of these trees. The USDA, Forest Service (FS) and others have developed risk maps based on data such as climate, frequency of importation of nursery stock, and forest cover type. Preliminary surveys in seven eastern states where risk was considered highest by the Forest Service were negative for *P. ramorum* in 2003. Surveys expanded in 2004 in thirty-seven states and in thirty-nine states in 2005. As of this date, these surveys have detected a forest infestation in California, San Francisco County in 2004.

Based on current information, we understand that *P. ramorum* requires wet or moist conditions, moderate temperatures, and living plant hosts to become established. Its spores can be found in soil and water as well as plant material and other articles. The risk of pathogen establishment and spread is greatest during rainy weather where host plants that support spore production are present. *P. ramorum* may be transported to new areas when infected plant materials and other infested material are moved.

In February 2002, the USDA, APHIS issued a federal domestic regulation for interstate movement of *P. ramorum* (7 CFR 301.92). The complete text, containing the list of

regulated and restricted articles and approved mitigation measures, may be found at APHIS' web site at http://www.aphis.usda.gov/ppq/ispm/pramorum.

In 2003, *P. ramorum* was detected in nurseries in British Columbia, Washington, Oregon and in California's Central Valley. These incidents catalyzed the need for a standard protocol for use by state and federal regulators to respond to new finds of *P. ramorum* in nurseries outside of the regulated area. To ensure that there is consistency in dealing with this disease, the "Confirmed Nursery Protocol" was developed. It describes the activities performed by APHIS staff and state agriculture regulatory officials to respond to new infestations by *P. ramorum* in nurseries. The nursery protocol may be viewed at http://www.aphis.usda.gov/ppq/ispm/pramorum.

1.1 STATEMENT OF NEED

This protocol was developed to address the possible occurrence of *P. ramorum* in forest and wildland settings beyond the quarantined and adjacent areas (currently 14 California counties and part of Curry Co., Oregon). It provides guidance to states, Federal land managers, and private landowners for deploying a rapid response to eradicate, suppress or otherwise contain the pathogen.

1.2 OBJECTIVE

The goal of this response protocol is to ensure that any and all infections or infestations by this pathogen are eradicated or mitigated. Landowner, agency and community co-operation is essential. Early detection and rapid reporting of potential *P. ramorum* infections are critical to ensure that spread is contained. The strategies employed in this response protocol are consistent with those employed in California where suppression, and Oregon where eradication are being carried out in forested areas.

2. ACRONYMS

APHIS	Animal and Plant Health Inspection Service
CEPM	Committee of Experts on Phytosanitary Measures
CFR	Code of Federal Regulations
CPHST	Center for Plant Health Science and Technology
DNA	Deoxyribonucleic Acid
EAN	Emergency Action Notification
ELISA	Enzyme-Linked Immunosorbent Assay
EPA	Environmental Protection Agency
ESA	Endangered Species Act
FAO	Food and Agriculture Organization
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FS	Forest Service
FWS	Fish and Wildlife Service
NAPPO	North American Plant Protection Organization
NEPA	National Environmental Policy Act
NMFS	National Marine Fisheries Service
ODA	Oregon Department of Agriculture
ODF	Oregon Department of Forestry
OSU	Oregon State University
NOAA	National Oceanic and Atmospheric Administration
PCR	Polymerase Chain Reaction
P. ramorum	*Phytophthora ramorum*
SPHD	State Plant Health Director
SPRO	State Plant Regulatory Official
USDA	United States Department of Agriculture
WG	Working Group

3. DEFINITIONS

Hosts and associated Plants: Plants listed in the "APHIS List of Regulated Hosts and Plants Associated with *P. ramorum*". Hosts have been found associated with *P. ramorum* and have had Koch's postulates completed, reviewed and accepted by APHIS. Associated plants have been observed with symptoms of *P. ramorum* and *P. ramorum* has been isolated and/or *P. ramorum* has been identified using polymerase chain reaction (PCR) Deoxyribonucleic Acid (DNA) testing.

Confirmed infected plants: Plants confirmed by an APHIS approved laboratory as being infected with *P. ramorum*, based on isolation of the causal organism or other APHIS approved diagnostic tests.

Delimitation survey: Survey conducted to establish the boundaries of an area considered to be infested by or free from a pest. In practice, this is a thorough investigation to determine the boundaries and extent of an infestation. Visual inspection, appropriate sampling and testing combined are used to map pathogen distribution.

Forest: A dense growth of trees, plants and underbrush covering a large area.

Free from: A consignment, field or place of production, without pests (or a specific pest) in numbers or quantities that can be detected by the application of accepted phytosanitary procedures. (FAO, 1990), FAO-CEPM, 1994.

Generally infested area: An area that has been determined to have an established *P. ramorum* population that is considered too extensive to eradicate or suppress. Also, an area under federal quarantine is considered generally infested as there is commonly no control of movement of infested material within that area and commonly no active survey conducted and reported to indicate otherwise.

Regulated area: An area into which, within which and/or from which plants, plant products and other regulated articles are subjected to phytosanitary regulations or procedures in order to prevent the introduction and/or spread of quarantine pests or to limit the economic impact of regulated non-quarantine pests. Federally regulated

	counties and areas identified in the *P. ramorum* regulations can be found at http://www.aphis.usda.gov/ppq/ispm/pramorum.
Regulated host:	Regulated hosts are species that show symptoms of *Phytophthora ramorum* under natural or nursery conditions (not inoculated). Also *P. ramorum* must have been isolated from the plant, confirmed via PCR, and Koch's postulates completed, documented and reviewed.
Presumed positive plant material:	Plants with visible symptoms of *P. ramorum* infection; and/or plants that have tested postitive using PCR or cultural isolation, but have not been confirmed positive for *P. ramorum* according to APHIS procedure.
Occurrence:	Presence in an area of a pest, officially reported to be indigenous or introduced, and not officially reported to have been eradicated. (FAO, 1990), FAO-CEPM, 1994. Term not used in this document, but definition provided to provide clarity to the term "outbreak" below.
Outbreak:	An isolated pest population recently detected and expected to survive for the immediate future. FAO-CEPM, 1994.
Quarantine area:	An area within which a quarantine pest occurs and which is being officially controlled. (NAPPO, 1985) FAO-WG, 1995.
SPHD:	The State Plant Health Director of a particular state. Lead APHIS contact in each state responsible for overseeing all Plant Protection and Quarantine activities in that state.
SPRO:	State Plant Regulatory Official. State Employee recognized as the counterpart to the federal SPHD. Contact person and lead state representative for overseeing all plant quarantine regulatory activities in that state.
State forester:	Head of State Forestry organization, in most States one of the agencies conducting forest surveys for *P. ramorum*.
Urban forest interface:	Area where structures and other human development meet or intermingle with undeveloped wildland.
Wildland:	An area where land is covered mainly by native vegetation. This does not include agricultural, urban or industrial areas. (Compare with "Forest")

4. ACTIVITIES NEEDED PRIOR TO AN OUTBREAK

4.1 SURVEILLANCE AND NETWORK DEVELOPMENT

Successful pathogen surveillance depends on a network of Forest Health Protection specialists, State forestry and State Department of Agriculture personnel, and university contacts that collaborate at many levels for detection, accurate diagnosis confirmed by APHIS and a coordinated rapid response. Any of these professionals, as well as arborists, gardeners, nursery owners, and the general public, may be the first to detect a new infection by *P. ramorum*. See "Preparing for Invasive Species Outbreaks: A Workbook for State Foresters", published by the National Association of State Foresters and posted at http://www.stateforesters.org/pubs.html.

4.1.1 Systematic Detection Surveys

APHIS has determined that *P. ramorum* is a pest of concern sufficient to initiate a quarantine in parts of California and Oregon and nursery and forest surveys. The USDA, FS Forest Health Monitoring Program has established a protocol for systematic survey of *P. ramorum* in forest environments. Thirty-seven states participated in this program in 2004 and thirty-nine for 2005. Detection, Monitoring and Sampling methods are described in Appendix A. These surveys may detect a forest or wildland infestation. APHIS has established a protocol for systematic survey for *P. ramorum* in nursery settings. All states participated in this survey in 2004 and 2005. Survey details are available on line at the APHIS P. ramorum web site.

4.2 TRIGGER EVENTS FOR REGULATORY ACTION

Suspect positives detected in forests and wildland areas outside the currently quarantined area and more than twenty-five miles from a generally infested area trigger immediate Federal notification and State action.

A suspect positive is: The detection of *P. ramorum* (by a State laboratory only, via PCR or isolation) from soil, water or vegetative material. Suspect positives must be confirmed by an APHIS laboratory to trigger a federal regulatory response.

Pending confirmation and depending upon their authority, States may initiate a regulatory hold on plant material surrounding the site, or negotiate a voluntary hold with landowners until federal confirmation can be obtained. Initiate notification procedures as appropriate (see Section 5).

Where sufficient authorities exist, an immediate response can be invoked by the State regulatory agency, which is similar to the anticipated Federal response by a detection of a confirmed positive:

- Harvesting, collecting or movement of the genera of host plant material in the area should be placed on hold. A quarter-mile buffer area (160 acres) is suggested until a delimitation survey can be completed.
- Equipment contaminated with soil will be cleaned of soil prior to movement to other sites (see Appendix C).
- State regulatory officials will coordinate management of access to the area to the degree possible and practical. Access should be restricted as much as feasible to minimize site disturbance and potential pathogen spread.

For details on holds and quarantine actions, see Section 6.

A confirmed positive is: The detection of disease caused by *P. ramorum* confirmed by USDA, APHIS laboratories in Beltsville, Maryland.

A federally confirmed detection will trigger a state or federal regulatory response. Pending the completion of a delimiting survey, (see Regulatory Action below, Section 6) for appropriate actions.

- Harvesting, collecting or movement of the genera of host plant material in the area should be placed on hold. A one-quarter mile buffer area (160 acres) is suggested until a delimitation survey can be completed.
- Equipment contaminated with soil will be cleaned of soil prior to movement to other sites (see Appendix C).
- State and Federal regulatory officials will coordinate management of access to the area. Access to the area will be restricted to minimize site disturbance and potential pathogen spread (see Section 6).

Upon completion of a delimitation survey, the size of the buffer area can be adjusted, either increased or reduced. An eradication or suppression program should be implemented (see Section 6). Plant material not of host or associated hosts may be released, unless determined to present a risk or found to be infected.

4.2.1 Identification of Regulatory Authorities

States with quarantines for *P. ramorum* have specific responsibilities and specific authorities, as authorized by their laws and regulations, thus specific actions within and around the infested area are expected to be conducted by the State personnel. For example, in Oregon, the Oregon Department of Agriculture (ODA) quickly adopted its own quarantine when *P. ramorum* was discovered in Curry County. The notifications to affected landowners were then based on State authority, using State forms. The Oregon Department of Forestry has no regulatory authority, but helped ODA delimit, treat and monitor the eradication projects. Support was provided by USDA, APHIS and USDA, FS.

In states without quarantines for *P. ramorum*, state regulations must be put in place to facilitate authorization for the actions determined to be necessary to appropriately

respond to the situation and to allow less than state wide federal quarantines, should a quarantine be necessary.

5. NOTIFICATION

5.1 IMMEDIATE NOTIFICATION OF SUSPECT POSITIVES

Field samples are sent for testing to designated plant disease laboratories. These laboratories will immediately communicate suspect positive finds as soon as one of the following has occurred:

- A positive PCR test.
- A culture that matches the morphology of *P. ramorum* (i.e. isolation of *P. ramorum*).

Notify the SPHD and the SPRO of the State (see lists of names, addresses and phone numbers at http://www.aphis.usda.gov/ppq/searchpage.html). The SPRO shall notify the state management agencies and the submitter of the sample.

Submit DNA samples for confirmation to the USDA Beltsville Plant Germplasm Laboratory located in Beltsville, MD (telephone: 301-504-8785) or an approved alternative laboratory. Submit culture samples for confirmation to the USDA National Identification Services located in Beltsville, MD (telephone: 301-504-5327). See the APHIS *P. ramorum* website for "Protocols" and "Diagnostics" for more specific information at http://www.aphis.usda.gov/ppq/ispm/pramorum.

If confirmed by APHIS, immediately notify the State Forester and the USDA, FS Forest Health Protection Regional or Area Director.

APHIS, PPQ Regional Office will notify the SPHD. The SPHD will be responsible for notifying any facilities that are impacted by material shipments.

Notify other state and federal partners, such as the State Extension Service Specialists, as appropriate.

5.2 ADDITIONAL NOTIFICATIONS TRIGGERED BY APHIS CONFIRMATION

All persons listed in 5.1 should be notified of the confirmation. Adjacent landowners should be notified, and depending on the local ownership patterns, may need to be asked for permission to enter their property to complete the delimitation survey. Consult your state's private property laws for guidance.

Headquarters will notify the appropriate staffs including the Phytosanitary Issues Management Team and the Center for Plant Health Science and Technology. APHIS Headquarters will also notify the North American Plant Protection Organization (NAPPO) through the Center for Plant Health Science Technology (CPHST) and

other international partners when an area is placed under restrictions or released from regulations, i.e., declared free from infestation.

Public notification: The SPHD and SPRO will use state channels, including public affairs offices to make any public announcements. The SPHD will insure that the appropriate Legislative and Public Affairs offices are aware of the pending release, via the Regional Office and National Headquarters Office. When possible and appropriate, the USDA, FS will work in conjunction with APHIS and the state agencies in public notification efforts.

Notifications provided to other States: As soon as possible, owners or managers of infested forest lands will work with their SPHD and SPRO to provide notification of any previous shipments of plant materials (logs, branches, leaves of hosts/associated hosts), to the receiving SPHD and/or SPRO. Details of any host shipments or associated host propagative shipments will be provided for a one-year period preceding the *P. ramorum* detection.

6. REGULATORY ACTION

6.1 HOLDS, LIMITED OUTBREAKS AND QUARANTINE ACTIONS

Measures must be initiated to prevent movement of infected plant material within or from a site where the pathogen is detected. All movement of plant material will be held (stopped) using the state equivalent of the APHIS Emergency Action Notification (EAN) – a document that notifies, in this case, a land owner of holds and controls to be put in place until a delimitation survey is complete. During delimitation, this hold will include hosts, associated hosts, and any other product or article that an inspector determines to present a risk of spreading *P. ramorum* from within the infested site. This clause allows flexibility in case suspicious symptoms are found on potential new hosts.

The appropriate regulatory response will depend upon the origin and extent of the infestation. If the pathogen has been recently introduced, and its distribution remains limited to material recently planted, the appropriate response may be to regard this as a "limited outbreak", with resulting action similar to that undertaken following a finding of *P. ramorum* in a nursery setting. In nurseries, all hosts and associated hosts contiguous with the infected host are destroyed until a 2-meter break occurs in host material. A 10-meter radius surrounding that is placed on hold for at least a 90 or more day period.

Where the infestation is larger, with secondary spread having occurred from the original source, a more rigorous response is appropriate. For an established infestation, a one-quarter mile (160 acre) buffer area around the known infected material should be put on hold, pending a delimitation survey. Equipment on site and within the one-quarter mile buffer will be properly cleaned and/or decontaminated prior to being moved (see Appendix C).

6.2 EVALUATE ORIGIN, EXTENT, SEVERITY, AND POTENTIAL IMPACT

These activities will be performed by the relevant regulatory personnel, usually the State Department of Agriculture. The State Forestry Organization should be asked to provide forestry expertise, as needed.

6.2.1 Delimiting Survey

All trees, shrubs, vines, and herbs (plants) in the vicinity of the infected plants should be visibly inspected, focusing on all host/associated genera, but keeping in mind that the host list continues to expand. Submit vegetation samples to the appropriate State or other APHIS approved laboratory for diagnosis. Sample those plants which appear unhealthy. See Appendix A: "Monitoring and Sampling" for examples and guidance. Survey is to cover the continuous forest type, at a minimum of 100-meters beyond the last symptomatic plant. In forest and wildland settings, the disease may be patchy. An early detection survey should be conducted outside the buffer zone. Roadside surveys or transects may be used depending on the local conditions. Roadsides, trails and landings should be observed for symptoms. In California and Oregon helicopters and aircraft are effective due to the presence of the tanoak, an indicator plant that is commonly killed by *P. ramorum*.

6.2.1.1 Determine the origin of the infestation

Determine links to host plants, related plants and other associated plants. Check soil, water and vegetation movement. Interview local residents and determine traffic patterns, activities and disturbances, and climate patterns. Site history, including soil, water, and management prescription, maintenance and other activities should be documented as completely as possible.

6.2.1.2 Trace forwards

Initiate trace forward investigations if at risk plant materials or forest products (e.g. logs, branches, or leaves) have been removed from the site. Transports made prior to the discovery of *P. ramorum* shall be identified and the SPHDs and SPROs of the receiving states shall be notified of all transfers made within the prior 12-months.

6.2.1.3 Soil

Determine soil type and history. Bait soil samples to determine if soil has been infested, using APHIS approved methods (see http://www.aphis.usda.gov/ppq/ispm/pramorum). If soil has been recently moved off-site, trace and inspect new locations for symptoms.

6.2.1.4 Water

Bait up and down streams of the area to determine if the pathogen is present in the water courses. For APHIS approved protocols see

http://www.aphis.usda.gov/ppq/ispm/pramorum. Note: if an irrigation system is in use, check for areas of standing water. Determine if water from the area is or has been used for dust abatement, fire fighting or other activities.

6.2.1.5 Debris piles

If trees or plants were recently removed from the area, check the area surrounding any debris piles for symptomatic plants, sample surrounding soil and bait for *P. ramorum*.

6.2.1.6 Equipment

If equipment used on the property is shared with others or transported to other areas, trace and inspect those areas.

6.2.1.7 Fungicides

If this is a urban forest interface setting, fungicides used on the site could hinder the detection of *P. ramorum*. If fungicides were used, record the date, type of material, amount, and application rate.

6.2.1.8 Soil amendments

If this is an urban forest interface, determine if any organic soil amendments were applied.

6.3 EVALUATE AND DEVELOP TREATMENT OPTIONS

If eradication is undertaken, the quarantine area will remain limited to the infested area and a buffer zone. As mentioned in Section 6.1, "Holds, Limited Outbreaks and Quarantine Action", the size of the appropriate quarantine area will depend upon the extent of the infestation. Limited outbreaks such as contained, recent introductions usually result in a much smaller quarantine zone than infestations that have established in the environment by spreading from infected planting to established plants in the landscape. Evidence of secondary spread should be considered in this decision. In Oregon, the quarantine zone under eradication was established at least one-half mile beyond the farthest infected plants, but this could vary depending on terrain, climate conditions and other factors.

Suppression projects are undertaken when eradication is not feasible. The goal is to control the spread, rather than to eradicate the pathogen. This would result in a larger area, such as the entire county, becoming quarantined. Should the state not implement regulations in the area that are "substantially the same" (note that these may also be more restrictive) for intrastate movement as are the Federal regulations, the entire state will be subject to quarantine.

6.3.1 Conduct Appropriate NEPA, ESA and FIFRA Assessments

When developing program actions for the control and management of *P. ramorum* the potential environmental effects of the proposed actions need to be considered. Any action, including but not limited to chemical treatment and host removal, must be given a hard look for potential environmental impacts.

There are several key federal environmental protection laws and implementing regulations that must be considered and complied with. These major laws include the National Environmental Policy Act (NEPA), Endangered Species Act (ESA) and Federal Insecticide, Fungicide and Rodenticide Act (FIFRA).

When considering a proposal for a major federal action under the NEPA, Federal agencies are required to prepare environmental analyses. The environmental analysis contains input other federal agencies, state and local governments, Indian tribes, and the public. Actions can be new or continuing activities, including projects and programs entirely or partly financed, assisted, conducted, regulated, or approved by federal agencies; new or revised agency rules, regulations, plans, policies, or procedures; and legislative proposals. An evaluation of an action may lead to the categorical exclusion that permits an action without further review, the development of an environmental assessment or a need for an environmental impact statement.

Section 7 of the ESA requires that Federal agencies consult with the U.S. Fish and Wildlife Service (FWS) and the National Oceanic and Atmospheric Administration, National Marine Fisheries Service (NOAA Fisheries Service) to ensure that any action authorized, funded, or carried out by the agency is not likely to jeopardize the continued existence of any Federally listed threatened or endangered species or result in the adverse modification of designated critical habitat. Consultation with FWS and NOAA Fisheries Service is initiated by the Federal agency conducting the action. Consultation begins with the action agency submitting a biological assessment of potential impacts and mitigations for threatened or endangered species and habitat which may be within program operational areas. Consultation can be formal, which results in a biological opinion from FWS and NOAA Fisheries Service and may take up to 120 days. Consultation can also be informal, which results in a letter of concurrence from the Service approving of the action agency's assessment of potential program impacts.

Under NEPA and ESA, every action must be reviewed for its potential impact on the environment and on threatened or endangered species by the program manager.

FIFRA regulates the use of pesticides in the United States. Only those pesticides registered for use by the Environmental Protection Agency (EPA) can be used in programs. These pesticides must be used only against the pests and in situations specified on the EPA approved pesticide label. In some cases, especially with new pests, the EPA can grant a program special permission to use unregistered products or registered products for uses they were not approved for.

In order to avoid delays in implementing program actions consider consulting early in the development process with environmental compliance staff in FS and APHIS. They can help determine how your program can best comply with these and other Federal, state or local environmental laws and regulations.

6.4 ERADICATION

Eradication measures require removal and destruction of all infected bole host material and of all foliar host plants and associated plants in addition to the surrounding buffer (see official list, the "APHIS List of Regulated Hosts and Plants Associated with *Phytophthora ramorum*", found at http://www.aphis.usda.gov/ppq/ispm/pramorum). This can be difficult in urban forest interface settings, where multiple landowners may be affected. Most SPROs have the regulatory authority to require treatment, but voluntary compliance is more preferable. For established infestations, a minimum 100 ft. buffer is recommended. Ensure destruction is carried out according to one of the methods detailed in Appendix B.

A regulated or quarantine area will be established around the confirmed infestation. For example, Oregon placed an area within one-half mile of the removed plants under quarantine with specific restrictions on plant movement from the quarantine area. Local topography and vegetation will need to be considered in the definition of the regulated area. The regulated area should be designed to halt or limit movement of potentially infested material off site.

See Appendix C, for basic sanitary measures that must be implemented immediately on all sites containing a positive plant. Land managers should utilize best management practices (see Section 6.5.1), but at the very least, tools and other implements should only be used in the destruction block or be disinfected prior to removal from the area. All plant parts removed from plants within the eradication and 100 ft. buffer area must be destroyed by an approved method (see Appendix B).

Treat stumps to prevent resprouting, as these may be reinfested and prevent successful eradication. In one site in Oregon where stumps could not be treated, repeated hand-pulling of sprouts was required to prevent reinfection of hosts and pathogen survival.

6.4.1 Monitoring

Conduct surveys at least once every spring, summer and fall until plant, soil and water surveys have been negative for a minimum of two-years. Survey must be done following leaf out and with adequate time for the pathogen to express symptoms. In Oregon, monitoring is done in late November/early December, in April, and in June.

Host plants, plant parts, soil, and other materials which may spread the pathogen may not be removed from the site or from any adjacent areas under quarantine, except for destruction, diagnostics or permitted research.

6.4.2 Conditions for Release

Areas which have been placed under regulatory control may be released from regulatory action by designated authority after two-years if the following has been demonstrated for two consecutive years:

- There are no additional detections of *P. ramorum* on site.
- Soil on the site and in the immediate area have tested negative for *P. ramorum*.
- Standing water and water courses in the immediate area have tested negative for *P. ramorum*.

Criteria for release of site and for phytosanitary certification of forest materials in the hold area will include use of appropriate diagnostic procedures as per APHIS protocol. The goal is to achieve an "area free from" status, which allows removal of all regulatory restrictions.

6.5 SUPPRESSION AND CONTAINMENT

If eradication over the entire area is not feasible, the State should consider putting as much of the area as possible under a suppression program, to slow the pathogen spread. Suppression of *P. ramorum* is based on the breaking of pathways of the pathogen or creating or utilitizing barriers (such as a host-free "firebreak") with the purpose of minimizing the likelihood of spread or survival of *P. ramorum* on the site, or its spread to new sites.

To accomplish this, it is recommended to destroy all confirmed plants/plant parts and adjacent symptomatic host/associated plants by an approved method. Removal of non-symptomatic host/associated plants in the surrounding area to the extent possible is also recommended. Treat stumps to prevent resprouting, if possible. Baseline and follow up monitoring, as done for eradication, should be conducted to determine program efficacy.

Under a suppression and monitoring program, a regulated area will be set up around the perimeter of the suppression project area. The regulated area will be much larger than the area surrounding an eradicated area, since the pathogen is not eliminated from the environment. The political unit of a county is expected, but a different regulated area might be appropriate under the right conditions and if agreed upon by Federal and State regulatory officials.

6.5.1 Best Management Practices

Mitigation measures to prevent the spread of *P. ramorum* from infested forest and wildland sites.

6.5.1.1 Risk of spread

The greatest risk for artificial spreading of *P. ramorum* is through the movement of infected plants or plant parts by individual actions. If infected plants are transported to a suitable environment with suitable hosts, the pathogen will likely become established over time. The pathogen survives in and can be spread via movement of infested soil and water. Aerial spread is thought to be limited to short distances of less than 100 meters, except perhaps during extreme storm events with strong winds accompanied by heavy rain.

Damp, humid conditions promote spore production and natural spread. Contamination of humans, animals, and equipment is greatest under these conditions. Detached plant leaves, organic material and soil, which may harbor spores of the pathogen, are likely to adhere to an individual, vehicle and/or equipment when damp or wet. Spread is more likely in settings where hosts of *P. ramorum* are common, such as forests and developed areas that are within or adjacent to areas of native vegetation.

The pathogen does not readily produce spores or spread naturally under extended dry conditions. Also, dry soil and organic material will not easily adhere to an individual or equipment.

6.5.1.2 Preventing spread

- Supervised crews working in an infested area should be informed that:
    - The area is infested with *P. ramorum* and the significance of that.
    - Unauthorized movement of plant material is prohibited.
    - Procedures to avoid contamination and prevent disease spread will be explained and must be followed.

- Do not collect or transport host plant material from an infested or quarantined area.

- Precautions should be taken to avoid becoming contaminated with the pathogen:
    - Avoid areas of concentrated disease to the extent possible.
    - Avoid entering infested areas during wet conditions.
    - Keep vehicles on paved and graveled surfaces when conditions are wet.
    - Stay out of areas of wet soil and mud.

When leaving an infested area, inspections should be conducted on individuals, vehicles and equipment for accumulations of mud, soil, organic material, and detached plant debris. Accumulations of these materials should be removed and decontamination procedures followed.

6.5.1.3 Removing contaminated mud, soil and organic material

The risk of spreading *P. ramorum* increases directly in proportion to the amount of accumulated mud, soil, organic material and leaves that is inadvertently transported out of an infested area. Crews should use sanitation kits to use for clean up prior to leaving

an infested site. Kits should contain a fresh chlorine bleach solution (10/90 mixture of bleach to water), scrub brushes, scrapers, gloves, towels, and plastic bags.

Use brushes and scrapers to remove heavy accumulations of soil and other debris from footwear. Rinse footwear with water to remove any remaining material. An additional level of sanitation is achieved by washing with soap and water or disinfecting with 10% bleach solution. Tools and any other items used in the clean up process need to be decontaminated. See biosecurity/sanitary methods posted at http://www.aphis.usda.gov/ppq/ispm/pramorum website.

Dirty clothes should be placed in a plastic bag until washed. Vehicles and heavy equipment should be swept out, making them free of soil, leaves and other plant debris prior to departing the site. Soiled vehicles and heavy equipment should be set up for a designated wash station before leaving the area. Wash water should be disposed of within the quarantine zone.

6.5.1.4 Establishing a power wash station for vehicles and heavy equipment

- Wash station should consist of:
  - A paved or rocked area.
  - A well drained surface so that exiting vehicles do not become recontaminated by the wash water.
  - Designed so that contaminants can be isolated, treated or disposed of properly. Contaminants may consist of wash water, displaced soil and/or organic debris.

6.6 NO ACTION ALTERNATIVE

Failure to eradicate or to attempt to contain an infestation within a county could result in a Federal quarantine on that county. Failure of a state to initiate regulatory action would necessitate a Federal quarantine on that entire state.

7. MONITOR TREATMENT EFFECTIVENESS

Areas will remain under quarantine for a minimum of two-years from the date of the last pathogen detection. Sites considered eradicated for *P. ramorum* will continue to be monitored for two years. If eradication has been successful, which has been defined as sites that remain pathogen free (plants, soil and water) for two-years post eradication, the sites will be eligible to be released from quarantine. See Appendix A for survey methods and guidance.

8. RESTORE AFFECTED AREAS

Take steps to stabilize soil, and to prevent off--+ site movement of the pathogen. Replant the site with appropriate nonhost plants. Avoid planting other members of any host genera and evaluate members of the same family of being a risk, as the host list continues to expand.

APPENDIX A

DETECTION MONITORING AND SAMPLING

Detection and monitoring protocols from the USDA Forest Service, National *Phytophthora ramorum* Survey of Forest Environments, the State of California and the State of Oregon are presented in Appendix A as examples, to display various methods used to address different local conditions and objectives. Select the relevant parts to customize a program for your area.

A.1 National *Phytophthora ramorum* Survey of Forest Environments

Steve Oak (soak@fs.fed.us) Posted at **http://www.fhm.fs.fed.us/sp/sod/sod.shtm**.

Objectives: Survey the forested perimeter of all nurseries receiving stock from infested nurseries; and survey at least one location in each high and moderate risk hexagon nationwide.

This survey is designed to gather information on the distribution of the pathogen *Phytophthora ramorum*, cause of the disease known as Sudden Oak Death. The survey strategy reflects the current understanding of the biology and ecology of *P. ramorum*, known hosts and potential hosts based on laboratory testing or taxonomic similarity, and likely pathways for its introduction. The best available science was used to determine appropriate risk factors and level of risk. As knowledge of *P. ramorum* host susceptibility, biology, and epidemiology changes or improves, the factors used in the risk assessment will also change.

The purpose of the survey is to detect the presence of *P. ramorum*. It is neither a population survey nor an attempt to express the amount of area affected by this pathogen. Additional delimiting and evaluation surveys will be done around newly detected infestations to estimate affected area and associated impacts.

RISK-BASED SAMPLING POLYGON DEVELOPMENT

Forest Health Monitoring has produced a National map identifying sampling polygons based on risk (Figure 1). Survey plot intensity will be stratified by risk ratings.

The following factors were used to assign risk and develop the sampling polygons:

1. Presence of known *P. ramorum* host species, host genera, and closely related genera.

   Overstory: Percent basal area of *Quercus* species in the red oak and live oak groups (eastern United States) or presence of known hosts (western United States).

Understory: Only the evergreen understory hosts were considered in this analysis. Presence of actual evergreen understory hosts (western United States) or number of evergreen host species (eastern United States).

2. Locations of nurseries receiving *P. ramorum* host stock.
   - ✓ Current analysis based on rhododendron stock.

3. Length of yearly mesic/moist weather period.
   - ✓ One, two, or three months with five inches of rain or three inches of rain and two days dense fog when temperature is between 60 to 80 degrees F.

4. Area outside limiting temperature extremes currently associated with *P. ramorum*.
   - ✓ Extreme temperature limits are defined as a minimum of one month with winter maximum temperature of less than 32 degrees F or one month with summer maximum temperature greater than 90 degrees F.

The smaller the polygon, the higher the perceived *P. ramorum* risk.

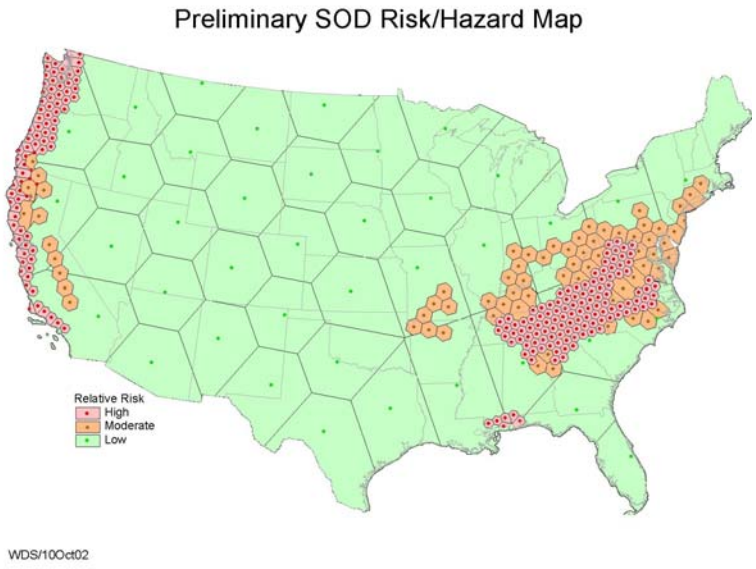


Fig. 1. Preliminary *P. ramorum* Risk Map

Risk as projected in Figure 1 can be modified by known or suspected importation of *P. ramorum* host nursery stock or other plant materials from infested areas elsewhere in the world. Where suitable host type vegetation occurs in proximity to such nurseries, risk is elevated.

FOREST SURVEY REQUIREMENTS

Sampling should be concentrated in high risk forested environments; however, a minimum sampling of low risk forest environments is encouraged if time and funding permit.

Polygon Sampling

Thirty locations is the target number of sample sites for each state participating in the survey. More sampling may be possible or less may be required, depending on the number of nurseries or other areas receiving potentially infested plant materials, budget constraints, etc. Detailed maps of risk polygons, road networks, and other landmarks are available by state in the form of ARCView compatible files from Steve Oak, USDA Forest Service, Southern Region- Forest Health Protection, Asheville, NC, soak@fs.fed.us.

Highest priority should be given to sampling in high risk polygons in forested areas adjacent to the nurseries that have received plant materials potentially infected with *P. ramorum*. In states where only a small number of nurseries are available for sampling, the remaining locations will be distributed among forested areas adjacent to other nurseries that are not known to have received such material, the general forest area, and lower risk classes. Sample sites should be geographically dispersed whenever logistically possible. Close cooperation with State Plant Regulatory Official (SPRO) in your state will be essential to determine the nurseries to be surveyed and other nurseries not receiving potentially infected materials but willing to be included in the forest survey, as well as in obtaining owner permissions for private land access.

Important: Some sampling of the general forest not associated with nurseries should be done in each state, if time and funding permit.

Forested areas around nurseries

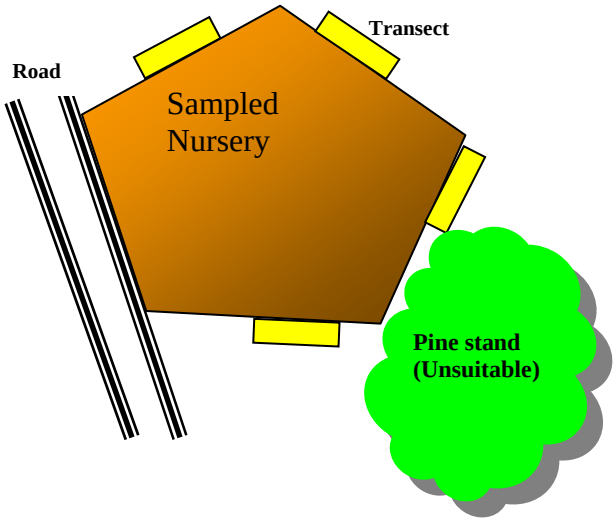
Sampling Scheme

Information concerning which nurseries should be surveyed is obtained through the SPRO in each state. The perimeter of the nursery with suitable host type adjacent will be identified and surveyed with four 100 meter transects distributed so as to sample all available aspects while ensuring that the microclimate most conducive to disease development (cool and moist) is surveyed. Thus, the plot location is selected based on the presence of attributes suitable for disease with regard to host type and environment (i.e., purposive rather than random). Suitable host type is defined as any forest type with a significant component of the following plant genera: *Acer, Aesculus, Castanea, Fagus Hamamelis, Kalmia, Lonicera, Quercus, Rhododendron, Vaccinium, and Viburnum.* Where available, give preference to forest types with a significant oak component. To be sampled as a nursery perimeter setting, this combination of overstory/understory must occur within 0.25 miles of the nursery production field. Sample the entire nursery perimeter if the total length is 400 meters or less.

On arrival at a nursery site, scout the nursery production areas for suitable host type and aspects to determine the particulars of available transect sites. This is not an area-based survey. Transect width is a function of the detection limit, i.e. how far you can see, which can vary according to the density of vegetation.

Target species/tissue types are:

Leaves:
Acer, Aesculus, Hamamelis, Kalmia, Lonicera, Rhododendron, Vaccinium, Viburnum
Bleeding Stem Canker:
Quercus, Castanea, Fagus



On the field form, record observer name, date, nursery perimeter setting, hex number, state FIPS code, county name, location name, and location number. Number locations consecutively. For each transect, record starting point witness tree information, starting and ending GPS coordinates (as well as any turning points with interim distances that may be necessary along the nursery perimeter), overstory composition, and understory species. Scout for hosts and symptoms while traversing the transect.

Collect any suspect bleeding stem cankers**,** distinguishing from seeps associated with stem boring insects, oak wilt, or other stem cankers. Carefully slice away outer bark to reveal symptomatic inner bark beneath with a drawknife or hand axe. Chip off a 5 X 5 cm section of bark that includes the active canker margin. Wrap sample in plastic wrap (e.g. Saran Wrap) and place in a plastic bag with a pre-printed label (provided) inside and out. Double bag all samples. Record state, hex number, transect number, location number and name abbreviation, host species code, and tissue type code on both labels and the field data sheet. Double bag host species separately if more than one is collected on a transect**.** Collect replicate samples on half of all transects at each location to be processed at the PCR lab at Mississippi State University as a quality control measure. While in the

field, keep samples cool on sealed coolant bags. Disinfect any tools used to scrape suspected stem cankers after each use and before moving on to a new candidate canker.

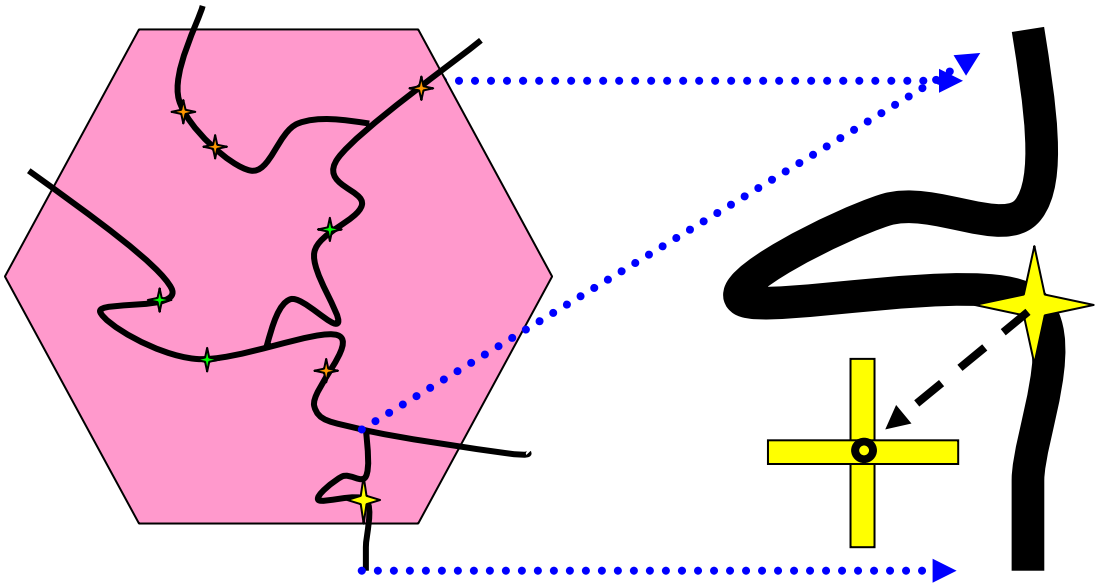
If foliar hosts are available, collect a maximum of 50 symptomatic leaves or shoots on each transect, keeping samples of each host species separate if more than 1 is available. Disperse sample collection along length of transect if symptoms are present throughout. Otherwise collect as available. Collect no more than 5 leaves from individual plants/clumps. When the length of the transect is traversed, examine all collected samples and select the 10 best symptomatic leaves of each host. Priority should be given to *Phytophthora* leaf and twig lesions if present, but do not limit collection to those. Recognize that most lesions will not resemble "typical" lesions caused by *Phytophthora* species. Place samples of each host in plastic bags with pre-printed labels (provided) inside and out. Double bag all samples. Record state, hex number, transect number, location number and name abbreviation, host type code, and tissue type code on both labels and the field data sheet. Double bag host species separately if more than one is collected on a transect. A clean, dry paper towel should be added to bags containing leaf samples to absorb excess moisture. Disinfect cutting tools used in leaf and twig collection before beginning sampling of a new transect. Collect replicate samples on half of all transects at each location to be processed at the PCR lab at Mississippi State University as a quality control measure.

Keep samples cool on sealed coolant bags while in the field and mail with any stem canker samples as soon as possible to the PCR diagnostic laboratory via overnight mail on sealed coolant bags in a special mailer (provided). Include a "Chain of Custody" form (provided) in a sealed plastic bag showing the sample ID's included in that mailing. It is permissible to collect samples on Friday so long as they are mailed overnight (i.e. for Monday morning arrival; maximum 72 hours after field collection). If Monday morning arrival cannot be guaranteed, then samples must be frozen until they can be mailed overnight the following week.

General forest areas not adjacent to nurseries

Although the sample location in forested areas not associated with nurseries is random, the plot location is purposive, i.e. the plot has attributes suitable for the disease with regard to host type. Once at a potential sample location, field crews will assess suitability of host type and the size of the area available for sampling. Travel to the next potential sample location if it's unsuitable. If it's suitable, walk off the road into the stand at least 100 meters to a plot center. On the field data sheet, record observer name, date, general forest area setting, hex number, state FIPS code, county name, location name, and location number. Number locations consecutively. Record GPS coordinates and witness tree data at the starting point, and install four 100 meter transects on cardinal azimuths from this plot center. The location of transects on the ground may be modified from cardinal directions to capitalize on optimal available host type and microclimate most conducive to disease. Use a compass to initiate the travel routes and the GPS unit to assist in navigation. This is not an area-based survey. Transect width is a function of the

detection limit, i.e. how far you can see, which can vary according to the density of vegetation.



<u>High-risk hex with road/forest type intersection.</u>

✧ Point where road network intersects forest cover .

✦ Suitable host type.

✧ Unsuitable host type.

✦ Suitable host type selected for field sampling.

<u>Road segment w/ sample point selected by field crew.</u>

Traverse from road point 100 meters minimum into suitable forest type to install plot center. Four sample transects are 100 meters long by 10 meters wide @ cardinal azimuths from plot center.

Fig. 2 Road/polygon intersection showing sample points location.

Traverse the entire length of the transect scouting for hosts and symptoms. Delay collection of leaf samples of foliar hosts, but collect any suspect bleeding stem cankers immediately; distinguish from seeps associated with stem boring insects, oak wilt, or other stem cankers. Collect samples as outlined above.

HANDLING PLANT SAMPLES

Collect samples as described above. Disinfect any tools used to scrape suspected oak stem cankers after each use and before moving on to a new candidate canker. Disinfect

other cutting tools before beginning sampling a new transect. Special care to disinfect boots or vehicles after sampling a location is required when surveying around a suspect positive location.

Keep different host plant species collected on the same transect in separate double bags from each other. Do not transport plant parts or bark samples unless double bagged, labeled inside and out and sealed, along with an accompanying "Chain of Custody" form. A clean dry paper towel should be inserted in bag with leaf samples to absorb excess moisture. Bark samples should be wrapped in plastic wrap, double bagged and labeled inside and out with preprinted labels (provided). All samples should be protected from direct sunlight and kept in a cooler on a sealed coolant bag or in a refrigerator until shipped. Mail samples to the appropriate diagnostic lab via overnight mail.

If samples cannot be mailed so as to be received by the diagnostic lab within 72 hours of collection, they should be frozen and mailed on sealed coolant bags as soon as practical. A completed chain of custody form should be sealed in a plastic bag and placed inside the container used to ship samples to the PCR diagnostic labs.

Laboratory Protocols

Diagnostic laboratories should report results to the Regional Forest Health Monitoring (FHM) Coordinators no less frequently than every 2 weeks. Regional FHM Coordinators should then forward those results to the SPRO, SPHD, and state forest health cooperator in the state where the samples originated; the APHIS National Program Manager (Jonathan Jones); the National *P. ramorum* Forest Survey Technical Coordinator (Steve Oak); and the National FHM Program Leader (Borys Tkacz).

Depending on the protocol and laboratory, false positive PCR results occasionally occur. In the case of an initial positive PCR result, the processing laboratory should repeat the reaction using surplus DNA from the initial extraction and/or from DNA extracted a second time from the stored sample. If the positive result persists after these tests, then the laboratory should contact the QA/QC laboratory (Dr. Susan Diehl, Mississippi State University) to determine if the sample has a replicate. If positive PCR results are accepted after consultation and retesting, inform the SPRO and SPHD in the state where the sample originated as well as the Regional FHM Coordinator. Confirmation of *Phytophthora ramorum* finds can only be completed by a diagnostic laboratory approved by APHIS. Therefore, repeat sampling of the transect yielding the positive sample will be necessary for additional plant samples to be used for culturing. Inform Dr. Laurene Levy (USDA-APHIS Beltsville, MD) of the pending shipment and send new samples.

A.2. California's General Guidelines for Conducting a Survey to Detect, Delineate, or Characterize *Phytophthora ramorum* Infection within an Area.

Prepared by Donald R. Owen, California Department of Forestry and Fire Protection. March 2003. Don.Owen@fire.ca.gov

California has forest lands in three categories: lands under quarantine, that are generally infested; lands under quarantine with a suppression program in place; and lands not infested. No single survey methodology will meet the needs of all situations. There are many considerations to be taken into account when designing a survey, such as the purpose, desired outputs and accuracy, attributes of the area to be surveyed, the data to be collected, time and personnel constraints, etc. Provided here are some general guidelines for conducting surveys on open-space lands with the purposes of:
1) Detection – determining presence or absence of *P. ramorum*,
2) Delineation – mapping disease distribution, and/or
3) Characterization – providing various descriptors of the disease and its impacts.

A survey may have more than one purpose and various methods may be used to achieve the desired outcomes. The methods described are intended to provide a general accounting of the disease situation on a given piece of land. They may be sufficient alone or may serve as a means of collecting baseline data that can be used for refining future surveys in the same or similar areas.

Basic Needs

- Familiarity with *P. ramorum* symptom recognition and sampling
- Map reading and orientation skills
- Map and aerial photo of the area to be surveyed – USGS 7.5 minute (1:24,000) topographic map and aerial photo of similar or larger scale.
- GPS Unit (use UTM NAD 83 coordinates) and compass
- Measuring tape or practice measuring distances by pacing
- Flagging, to mark the survey boundary, plots, routes of travel, etc.
- Data sheet
- Binoculars
- Camera for documenting symptoms, samples, etc.
- Supplies for taking diagnostic samples
- Supplies for cleaning boots and tools that may become contaminated by the *P. ramorum* pathogen

Preparation

- Describe survey's purpose
- Gather available information on the property – review maps and aerial photos, query the landowner and other sources of information
- Determine method(s) to be used and data to be collected
- Modify the data sheet, if necessary, to meet individual needs of the survey
- Prepare the map for field use – draw in the survey boundary, survey subunits, transect lines, plot locations, route of travel, etc.

- Prepare GPS for field use – load useful waypoints such as plot locations, boundary corners, endpoints of transect lines, etc.

Detection Survey

The purpose of a detection survey is to determine the presence of *P. ramorum* in an area where the disease is not known to exist. As a prerequisite, it should be confirmed that no disease has been reported for the immediate area. Maps of the confirmed distribution of *P. ramorum* are found on the *OakMapper* website, which can be accessed via a link from http://www.suddenoakdeath.org. In California, it may also be possible to obtain *P. ramorum* distribution information from the County Agricultural Commissioner's office and local offices of the University of California Cooperative Extension and California Department of Forestry and Fire Protection.

The best method for conducting a detection survey is to traverse the area following a series of parallel, evenly spaced transect lines, continually looking for disease symptoms as you walk. In effect, the area being surveyed is a strip that extends outward a certain distance on either side of transect lines. All known hosts are visually scanned for symptoms, both to the right and left of the transect line, within the boundaries of the strip. All transects should be walked in the course of the survey, but the surveyor should be willing to make deviations from survey strips in order to further investigate areas of specific interest -- for example, areas with a concentration of symptoms, areas that that are suspected to have an abundance of hosts but are not within the boundaries of the survey strip, etc.

The intensity of the survey will determine the likelihood of finding *P. ramorum* if it is present. Intensity can be measured as a percentage of the area that is visually inspected and will vary based on the width and spacing of survey strips. From a practical standpoint, the width of the strip is roughly estimated based on the distances to the right and left that a surveyor can effectively scan for symptoms. This obviously is not a precise measurement, but it does provide a means for determining the approximate area of land that has been visually surveyed. For small properties, e.g. less than 10 acres, it may be possible to survey close to 100% of the property. With larger properties this would be impractical. As a minimum standard for a detection survey, a 20% strip survey is recommended. Figure 1 illustrates how this could be achieved. Parallel transect lines are plotted on the map at 100m intervals. The surveyor uses a strip width of 20m (10m on either side of the transect line) and visually scans all hosts within the boundaries of the strip as the transects are walked. This example, i.e. 20m-wide parallel strips spaced at 100m, could be used with any size or configuration of land to achieve a 20% survey. The same width strips spaced at 50m intervals would achieve a 40% survey. Strip width can be varied to meet the preferences of the surveyor(s) or to better conform with site conditions, e.g. heavy vegetative cover may warrant narrower strips. If strip width is decreased, spacing between strips will also need to be decreased to maintain the same level of survey intensity. From a practical standpoint, 20m is about the maximum strip width that a single surveyor should consider using.

Topography can have significant influence on host and disease distribution. For this reason, it is generally best to plot transect lines roughly perpendicular to contour lines (up and down the slope). If lines are plotted parallel to contour lines, certain topographic features may be missed or poorly represented in the survey, i.e. ridgelines, stream bottoms, etc. It may not be necessary or practical to survey an entire property the same way. For larger properties in particular, the best approach may be to partition the property into more uniform subunits. Topographic maps, aerial photos, and other sources of information can aid in this process. Changes in topography may warrant changes in the orientation of transect lines. Changes in vegetative cover may warrant different survey intensities. Some areas may be devoid of hosts and be excluded from the survey, while other areas may be of lower or higher risk for disease based on the kinds and numbers of hosts present.

During the survey, closely inspect all symptomatic hosts to decide if a diagnostic sample is warranted. Determining the presence of *Phytophthora ramorum* requires lab confirmation. This is essential when conducting a detection survey in an area where the pathogen's presence is uncertain. Keep a record of *P. ramorum* hosts and symptoms encountered. Record the locations of symptomatic hosts and where diagnostic samples are taken, so that these areas can easily be returned to. Each sampling location should have a unique identifier.

Even with the most thorough survey, there is always the possibility the disease will not be detected when, in fact, it is present. It is also possible that symptoms will be found, but the pathogen cannot be detected in samples submitted for lab analysis. The best time for symptom recognition and sample collection will vary with climate type. The best time in California is during winter and spring. In Washington State, fall sampling is recommended. Also, the pathogen is more readily isolated from foliar samples, especially from California bay laurel (*Umbellularia californica*), than from bark and wood. All samples should be kept cool and processed as quickly as possible. Survey and sample collection can occur at any time of year, but the aforementioned factors may influence the outcome of the survey. Follow-up surveys may be warranted.

Delimitation or Delineation Survey

A delineation survey has a different purpose than a detection survey, but the basic survey methodology is the same, i.e. traverse the area following a series of parallel, evenly spaced transect lines while visually scanning all known hosts within a certain distance, both to the right and left, of transect lines (refer to the discussion under detection survey). The purpose may simply be to map the area-wide distribution (presence or absence) of disease, but more than likely additional information will be desired -- on which hosts is the disease present? What are the relative levels of infestation (high, medium, low, or absent)? Diagnostic samples may not be necessary if there is good evidence that disease already exists in the general area, in which case disease presence or level of infestation is inferred from the symptoms observed. (Diagnostic verification is needed for regulatory action to occur.)

The intensity of the survey will depend upon the desired accuracy and the relative abundance of disease. If a high level of resolution is desired and/or the disease is believed to be relatively rare, greater survey intensity is warranted, i.e. consider a strip survey of $\geq$ 20%. Also consider doing the survey in stages. For example, a 5-10% strip survey might be sufficient to establish the general disease distribution across the property. This could be followed by more intense surveys that are limited to particular areas of special interest. Meandering searches, i.e. those that do not follow transect lines, can be used to better define distribution patterns and their boundaries. As was described for the detection survey, the property can be partitioned in to more uniform subunits that are surveyed according to their particular attributes. A delineation survey need not follow a strict protocol; be flexible in designing a survey that best meets your needs.

Distribution data is best expressed as a continuum. A major advantage of a systematic strip survey is that it allows you to effectively sample a large area of land and to view conditions of interest as a continuum as you walk the property. If you are interested in a small amount of information, e.g. the distribution of symptomatic oak trees, it may be relatively easy to map the occurrence of each symptomatic and non-symptomatic oak within the boundaries of the strip survey. Collecting greater amounts of information, however, can be overwhelming and time consuming, e.g. attempting to map the occurrence of every symptomatic species of host plant. Keep in mind the purpose of the survey. Because you are doing a delineation survey, it is important that you continually observe conditions as you walk – this will enable you to better discern distribution patterns. Especially look for changes or unusual conditions and record them. Other data, which provides details to your overall observations, need not be recorded on a continual basis. You may decide to collect detailed data only at given points along the transect. To avoid bias, it is best to predetermine how this will be done. For example, every 40m along the transect, stop and record data on conditions within a given distance of your position.

If *P. ramorum* symptoms are known to exist in the survey area, diagnostic samples should only be taken to confirm potentially new or unusual occurrences of disease, e.g. symptoms on an unusual portion of a host plant, suspicious symptoms on a non-host, etc. If diagnostic samples are taken, record each sampling location and provide it with a unique identifier. You may also consider taking samples to determine if other pathogens, such as *P. nemorosa,* are in the area.

Characterization Survey

The purpose of this type of survey is to estimate parameters that relate to *P. ramorum,* e.g. what percentage of hosts are diseased, what percentage have been killed by the disease, what is the average age and diameter of diseased trees, etc. The procedure is to sample a subset of the population of interest and use the sample data to estimate population parameters. There are many different ways this can be done with no one best method for all situations. The sampling intensity, method used, and variability of the attributes being measured will influence the accuracy of the estimates. A discussion of

these considerations is beyond the scope of this document. A text on forest or vegetation sampling should be consulted for further information.

What is suggested here is a line-plot sampling method. No attempt is made to determine the statistical accuracy of parameters that are estimated by this method, but sampling intensity, i.e. the percentage of the area that is sampled, can be used as a relative measure of accuracy. The first step is to decide upon a sampling intensity, e.g. 5, 10, 20, 40 %, etc. Guidelines are available to aid foresters in making this decision for timber stands when the approximate density and distribution of trees is known, but there are no appropriate guidelines for areas with *P. ramorum*. If *P. ramorum* is common and host distribution is fairly uniform across the area being sampled, a lower sampling intensity may be sufficient. Partitioning the area into more uniform subunits can be helpful.

The next step involves deciding upon plot size and calculating the number of plots needed for a given sampling intensity. A common plot size used in forestry is one quarter of an acre (roughly $1/10^{th}$ hectare). The number of plots needed equals total area multiplied by % sampling intensity divided by plot size. For example, assume the total area of interest is 100 hectares. If a 5% sample is chosen, then 50 $1/10^{th}$ hectare plots are needed, i.e. (100 X .05)/.01 = 50. As in the previous sampling methods, a series of parallel, evenly spaced transect lines are established. Plots are distributed evenly along these lines. The total length of the transect lines divided by the number of plots equals the distance between plot centers. If circular plots are used, a ¼ acre plot has a radius of 58.9 ft. and a $1/10^{th}$ hectare plot has a radius of 17.84 m. All data is collected from within the plot boundaries.

A.3 Oregon Post-treatment *P. ramorum* monitoring protocol

Nancy Osterbauer, nosterba@oda.state.or.us [Note this document has been abridged, for the entire original document contact Nancy Osterbauer]

Phytophthora ramorum has been detected in a small part of one county in Oregon. The Oregon Department of Agriculture (ODA) issued a quarantine against *Phytophthora ramorum* to prevent further spread and to protect Oregon's agricultural and timber industries and natural resources (ORS 561.510 and 561.540). The following survey for *P. ramorum* has been implemented to assist in the detection and eradication of the pathogen. This post-treatment *P. ramorum* monitoring standard operating procedure is followed when surveying and processing samples from known positive sites in Curry County, Oregon for the pathogen, *Phytophthora ramorum*. The eradication efforts in Curry County, Oregon are being conducted in a joint effort by the Oregon Department of Agriculture (ODA), Oregon Department of Forestry (ODF), Oregon State University (OSU), and USDA Forest Service (FS). ODA laboratory personnel are responsible for collecting post-treatment plant and soil samples from within positive sites. Post-treatment samples shall be processed and results recorded by trained ODA personnel. A plant pathologist shall make final identification of *P. ramorum*.

SAMPLE COLLECTION AND SAMPLE HANDLING

The *P. ramorum* survey will be conducted every Winter, Spring, and Summer for 24 months after the initial eradication treatment. All oak and tanoak samples collected must be processed in the field. All other samples should be processed in the field whenever possible. Samples will be delivered to the laboratory in sealed plastic bags (e.g. Ziploc bags) in a cooler and then processed within 48-hours upon arrival. Plates should arrive sealed and in a cooler. Samples without a sample submission form will not be accepted. Aseptic technique will be used with all sample plating. Plated samples will be stored in the absence of light at room temperature.

Footwear must be sprayed thoroughly with a 10% bleach solution after each site inspection to avoid spread of the pathogen. Vehicle tires must be washed clean of soil before leaving the area. All field and laboratory tools must be sanitized/sterilized after each use. All samples and plated samples must be sterilized in the autoclave at 121˚C and 15 psi for 30 minutes at the conclusion of the survey.

PROCEDURE

Visually survey each treated site for host plants symptomatic for *P. ramorum.* Use Table 1 to determine the acreage to be visually inspected and then run a transect survey through each treated site. The number of plant and soil samples to be collected from each treated site is also listed in Table 1. Determine the transect to be followed. Survey as much of the treated site as possible within the guidelines given in Table 1. Examine all host plants within the transect for *P. ramorum*-like symptoms. Collect host and soil samples while walking the transect(s). To determine how often to collect a sample, divide the length of the transect by the number of samples (host and soil) to be collected. Using Example 1 (8.6 acre treated site) and the information given in Table 1, a host sample would be collected every 24 yd along the transect and a soil sample every 48 yd. Soil samples need only be collected during the Winter and Spring survey periods.

Collect plant samples from hosts with suspicious symptoms. If no symptoms are present, collect samples from asymptomatic hosts. Mark the location of the host with GPS. Record this information and a description of the sample on the sample submission form. Assign each plant and soil sample a unique number. Label the host with yellow flagging and an aluminum marking tag. Write the sample number and date on the flagging and tag.

During the Winter and Spring survey periods only: Collect soil samples at the base of host plants (see Table 1 and Host Specific Sampling Section 6.2). Preferentially collect soil samples at the base of symptomatic hosts. If no symptoms are present, collect samples at the base of asymptomatic hosts. Mark the location of the nearest host with GPS. Record this information and a description of the sample on the sample submission form. Label the nearest host with an aluminum marking tag. Write the sample number and date on the tag. Assign each soil sample a unique number. Wash the soles of your

shoes and your tools with a 10% bleach solution using a hand-held sprayer before leaving the area.

Table 1. Sampling table for examination of treated sites for *Phytophthora ramorum*.

Size of Treated Site (acres)	Area visually inspected (%)	No. of Plant Samples Collected[1]	No. of Soil Samples Collected[1,2]
0.00 – 1.00	80.0	8	4
1.01 – 1.25	72.0	10	5
1.26 – 1.50	67.0	12	6
1.51 – 2.00	55.0	16	8
2.01 – 2.50	48.0	20	10
2.51 – 3.00	43.3	24	12
3.01 – 4.00	35.0	32	16
4.01 – 5.00	30.0	40	20
5.01 – 6.00	26.2	48	24
6.01 – 7.00	23.6	56	28
7.01 – 8.00	21.9	64	32
8.01 – 9.00	20.6	72	36
9.01 – 10.00	19.5	80	40
10.01 – 50.00	4.0	80	40
50.01 – 100.00	2.5	80	40
100.01 or more	2.0	80	40

[1]See Host Specific Sampling (Section 6.2) for more information. This number represents the minimum number of samples taken.
[2] Soil samples will be collected only during the Winter and Spring survey periods. During the Summer survey period, only plant samples will be collected**.**

A post-treatment survey will be conducted three times each year, once in the Winter (late November or early December), once in the Spring (late March or early April), and once in the Summer (June). If the site remains free of *P. ramorum* for 24-months post-treatment, the pathogen will be considered eradicated. If the pathogen is detected, the site may be treated again and subject to the survey and monitoring previously described. During re-surveys, it is preferable to cover different transects across the site.

Host Specific Sampling: Oaks and Tanoaks

P. ramorum-killed trees are often infested by the western oak bark beetle *(Pseudopityophthorous pubipenni*s), oak ambrosia beetle *(Monarthrum scutellar*e), and the wood rotting fungus, *Hypoxylon thouarsianum*. The presence of bark beetles and *Hypoxylon* does not clearly indicate *P. ramorum*. However, as frequent associates, they may serve as valuable identification tools. Samples must be plated onto PARP medium in the field. Begin by shaving away the outer bark approximately 3-6" above or to the side of a seeping area. Shave away the bark in the area of the lesion until a canker margin or zone line is evident. Do not shave into the xylem. Use a knife (or scalpel) and

forceps to excise small pieces (approx. 1/8" or smaller) of the phloem. Include both healthy and necrotic phloem tissue on both sides of the zone line. Insert eight to 10 pieces firmly into the PARP medium. Seal the plate with tape or parafilm and label with the date, sample number, and host species. Fill out the sample submission form, including GPS coordinates for the sample. Sanitize all tools (e.g., axe, knife) with the 10% Clorox solution before proceeding to the next sample. Place the plate(s) and sample submission form(s) in the cooler and deliver to the ODA Plant Health Laboratory as soon as possible. Samples without a sample submission form will not be accepted and will be autoclaved immediately upon arrival. Sanitize the cooler with a 10% bleach solution for 20-minutes before re-use.

Rhododendrons and other foliar hosts

Along the survey transect, examine foliage (especially young leaves) for leaf spots, stem cankers, and/or dieback. If time and weather permit, plate samples on PARP in the field. Preferentially collect samples from diseased tissues. Using a sanitized knife or pruning sheers, isolate from the disease margin. If no disease margin is present, isolate from the leaf tip or the petiole of the leaf. For Oregon Myrtle, (California bay laurel) isolate only from the leaf tip. For tanoak, isolate only from the petiole. Insert eight to 10 sample pieces firmly into the PARP medium. Label the PARP plates with the date, sample number, and host species and seal with tape. Fill out the sample submission form including GPS coordinates for the sample. Sanitize all tools (e.g., knife, pruning sheers) with the 10% bleach solution before proceeding to the next sample. If time or weather prevents plating, place the collected plant tissues in a ziploc bag(s). Label the bag with the date, sample number, and host species. Fill out a sample submission form including GPS coordinates for the sample. Sanitize all tools before proceeding to the next sample. Isolate from the samples as soon as possible (within 48 hours). After isolation, plant samples must be sterilized (autoclaved) prior to disposal. Place plates and/or sample bag(s) in the cooler and deliver to the ODA Plant Health Laboratory as soon as possible. Plates and/or samples without sample submission forms will not be accepted and will be autoclaved immediately upon arrival. Sanitize the cooler with a 10% bleach solution for 20-minutes before re-use.

Soil Samples

Soil samples will be biologically biased towards successful isolation of *P. ramorum*. Soil samples will be collected at the base of symptomatic host plants or near the stumps of treated (removed and burned) host plants. Soil should be collected from three locations surrounding the host plant and/or stump. Collect a total volume of ~500 ml (~0.5 quarts) of soil in a Ziploc bag. Label the bag with the date, sample number, and nearest host plant. Fill out a sample submission form including GPS coordinates for the nearest host plant. Sanitize all tools before proceeding to the next sample. Place sample bag(s) in the cooler and deliver to the ODA Plant Health Laboratory as soon as possible. Sanitize the cooler with a 10% bleach solution for 20-min before re-use.

APPENDIX B

TREATMENTS

B.1 OREGON ERADICATION TREATMENT PROTOCOL

After initial confirmation of *Phytophthora ramorum* in a forest setting, plants in the vicinity are surveyed for symptoms. Understory hosts are checked for dieback, cankers, and leaf spots. Stems of host trees are inspected for bleeding cankers. The treatment area boundary is then flagged to include all plants positively identified for *P. ramorum*; those host plants with diagnostic symptoms for *P ramorum* and a 100-foot buffer zone from the last symptomatic plant. A 50 ft. buffer has been shown to be insufficient. The buffer zone size varies based on natural breaks in vegetation type, location of roads, and changes in terrain.

All host plants are cut and piled on site. Where possible, a stump-top application of herbicide to prevent resprouting is done immediately after cutting.

Piles are burned as soon as possible. Broadcast burning to consume leaf litter in the treated area is also done when possible. If sites are small and broadcast burning is not feasible, litter is raked into piles and burned.

Machinery, tools, and boots used in treated areas are disinfected or washed prior to leaving the site.

Follow-up removal of any new host sprouts is done where herbicide applications are not feasible. Sprouts are removed manually, piled, and burned on site.

B.2 TREATMENTS FOR MATERIAL DESTRUCTION

Incineration (burning to ash)

Burning may be through open burning or in an incinerator. Burn all infected plants, associated litter, and leaf debris in and around the site. Alternatively, these materials may be disposed of by incineration at a facility (location) approved by USDA and permitted within provincial and municipal statutes. Off site movement must be properly safeguarded and every effort to prevent plant debris or soil from being dislodged from the plants prior to incineration should be taken. A curtain burner may be used.

Deep burial

the infected plants, associated litter, and all leaf debris in and around the site must be placed in double plastic bags of 4 mil total thickness (two, 2-mil bags) or greater and buried to a depth of no less than two meters. The material must be buried at a USDA approved site, onsite, or at a municipal landfill which is expected to remain undisturbed. Every effort to prevent plant debris or soil from being dislodged from the plants should be taken.

B.3 Suppression Plan for Humboldt Co. , CA

Jack Marshall, California Department of Forestry and Fire Protection.
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Humboldt Co. is 350 miles north of the generally infested area in California. It has an isolated occurrence of *Phytophthora ramorum*. County wide aerial surveys (with ground-proofing) and numerous foliar samplings over past two years have yielded Redway as Humboldt County's only known infected site. Eradication was not considered practical in Redway, because the infestation occurred in an old-growth redwood forest, with many landowners affected. A suppression program was developed to slow the spread of *P. ramorum* in Humboldt County. The plan below outlines the logic and the action plan developed to meet this goal:

I. Assumptions

a) Suppression action is practical
b) Complete eradication is not feasible.
c) Suppression is feasible.
d) Not all infections will be found.
e) Not all landowners may participate in sampling/suppression activities. (This may depend upon each state's regulatory authority.)
f) If original infections arrived via wind or water, none of our actions will change the susceptibility of remnant hosts, given they lack genetic resistance. One action, which may alter susceptibility of Redway area, is to remove all *P. ramorum* hosts. However, not all *P. ramorum* hosts may be known at this time.
g) California bay laurel is both the primary host and the primary source of new infections in Redway.

II. Action Constraints

a) The option of underburning or pile burning to suppress *P. ramorum* population in litter layer was not feasible in the residential areas.
b) Use of inmate crews as labor force for host removal was not an option within residential area.
c) Availability of a feasible area in which to burn removed materials was limited.
d) Wildlife habitat restrictions (tree size, time of year, etc.) would be considered if operations extended into or abutted neighboring State Park property.
e) Potential for property destruction (houses, fences, landscape plants, autos, etc.) from tree felling.
f) Clearance with Utility Companies (power, phone, gas). On-site inspection with utility representative was conducted.
g) Only used certified or agency-trained tree faller.
h) Required written approval from landowners for sampling (foliar and soil) and host removal.

i) Ground raking and/or burning may have required restoration. Restoration plans were drafted, but not implemented.
j) Obtained a burn permit from County's Air Quality Control Board.

III. **Suppression Action for February 2004 Project**

a) Foliar samples
    1. Resample known infected trees and other symptomatic hosts flagged for removal.
    2. Surveyed hosts near proposed burn site (large clearing) prior to project. No symptomatic hosts were observed.

b) Soil samples
    1. Prior to tree removal, took soil samples from beneath the tree's drip line at each cardinal direction. With trowel, removed soil to a depth of 5 cm. Drip line samples were combined. Trowel was wiped clean and sanitized between samples.
    2. Soil sample "controls" were taken from a distance 5-to-10 meters from the drip line, and not from beneath other symptomatic hosts. The control samples were also combined within baggies. Kept samples in coolers for transport to UC Davis's plant pathology lab for analyses.
    3. Mapped tree locations and recorded drip line distances from stems so areas can be resampled at a later date.
    4. Also took pre-project soil samples from perimeter of assigned burn pile area, and from the drain's outflow area of Eel River Conservation Camp's wash station.

c) Tree removal
    1. Prior to beginning field portion of project, addressed crew with safety message.
    2. Known infected hosts were bay and redwood.
    3. For known infected bays and the lone redwood sapling:
        i. Felled known infected trees. Symptomatic seedlings were pulled from ground by hand.
        ii. Lopped all branches from bay trees; bucked and stacked bay stems on site. Known coast redwood sapling was not removed due to a lack of authorization from landowner. The tree was reinspected and no new symptoms were observed.
        iii. Placed and bundled bay branches on poly tarps and transported to stake side truck. If trees were near road, material hand carried to stake side truck (solid sides, not slats).
        iv. Removed symptomatic hosts within 50-foot radius stem.
        v. Raked ground litter for a distance of twice the crown radius of and beneath infected host. Also raked ground litter from fall zone of felled trees. For large raking zones, litter was transported to stake side truck using poly tarps. For smaller zones, material was bagged. Where raking was not practical (tall grass or ornamental beds of ivy), litter was scooped up by hand and bagged.

vi. Secured stake side load with canvas or poly tarps and took to designated site for burning (Eel River Conservation Camp dedicated a burn site and crew for burning).

IV. Sanitation Practices

a) Between properties and at end of project, sanitized boots, chain saws, rakes, pruning shears, etc. with 10 percent bleach solution. Had stiff brushes available to first remove accumulations of soil before spraying. Sanitation station was on a large poly tarp on ground, and adjacent to road pavement.
b) Vehicles leaving project area were washed at Power Station near lower Redway.
c) Stake side truck was washed at the local power wash as it left lower Redway for Eel River Camp to dump each load. At Eel River Camp, after downloading material from bed of truck, vehicle (including bed interior) was washed at the Camp's wash station before returning to Redway and at project's end.
d) At end of project, the sanitation station tarp was washed at the local power wash station in Redway. The power wash bay was then thoroughly washed to minimize off-site movement of soil and contaminants.

APPENDIX C

DISINFESTATION METHODS

Where it is necessary that visitors enter the site, every precaution should be taken to prevent off-site movement of infected plants, contaminated soil or debris. Plan ahead and be prepared with supplies.

If it is practicable, tools such as chain saws, axes, pruners, and other implements used in the project area should only be used on site. If tools and other implements must be moved from the site, then regular disinfection using an appropriate labeled disinfectant for the control of *Phytophthora* (such as 1/9 solution of chlorine bleach) is recommended prior to removal from the site.

Clorox bleach (sodium hypochlorite) is labeled (EPA Reg. No 5813-50) for treatment of water (~50 ppm available chlorine) for use against quarantine pests. The following methods were developed to control the spread of *Phytophthora lateralis* via water used for irrigation, dust abatement, fire suppression and equipment cleaning or other uses.

Everyone entering and leaving the site must scrape off loose pieces of soil. Those working with, or in contact with suspected infected material (including plants), must wash hands using soap, or disinfectant immediately after completion of task. A disinfectant footwear bath could be placed and used by personnel entering and exiting the infested site, where the movement of soil or plant debris on footwear is likely. The foot bath must be filled with fresh disinfectant on a daily basis. Do not visit other sites in potentially contaminated footwear or vehicles.

The tires (or other parts in contact with the soil) of vehicles must be cleaned of loose soil before leaving the infested site.

APPENDIX D

DIAGNOSTICS

Testing: The pathogen can be definitively identified by culturing or using PCR methodology. ELISA may be used to screen samples for the presence of *Phytophthora*, but is not specific for *P. ramorum*. Samples negative by ELISA testing may be discarded. While a State may initiate a hold based on a suspect positive, confirmation by an APHIS-approved laboratory is required for interstate regulatory action.

See **http://www.aphis.usda.gov/ppq/ispm/pramorum/surveyplan06/index.html** for methodology.

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APPENDIX F

APHIS LIST OF REGULATED HOSTS AND PLANTS ASSOCIATED WITH *PHYTOPHTHORA RAMORUM*

(Revision dated 11 September 2006)

This list is continually being updated.

The most current version is posted at: http://www.aphis.usda.gov/ppq/ispm/pramorum

Proven Hosts Regulated for *Phytophthora ramorum*

Scientific Name (47)	**Common Name(s)**	**Notes**
Acer macrophyllum	Bigleaf maple	
Acer pseudoplatanus	Planetree maple	Koch's postulates completed
Aesculus hippocastanum	Horse chestnut	Koch's postulates completed
Adiantum aleuticum	Western maidenhair fern	
Adiantum jordanii	California maidenhair fern	
Aesculus californica	California buckeye	
Arbutus menziesii	Madrone	
Arctostaphylos manzanita	Manzanita	
Calluna vulgaris	Scotch heather	
Camellia spp.	Camellia - all species, hybrids and cultivars	
Castanea sativa	Sweet chestnut	
Fagus sylvatica	European beech	
Frangula californica (≡Rhamnus californica)	California coffeeberry	
Frangula purshiana (≡*Rhamnus purshiana*)	Cascara	
Fraxinus excelsior	European ash	
Griselinia littoralis	Griselinia	
Hamamelis virginiana	Witch hazel	
Heteromeles arbutifolia	Toyon	
Kalmia latifolia	Mountain laurel	
Lithocarpus densiflorus	Tanoak	
Lonicera hispidula	California honeysuckle	
Laurus nobilis	Bay laurel	Koch's postulates completed

Maianthemum racemosum (≡ *Smilacina racemosa*)	False Solomon's seal	
Michelia doltsopa	Michelia	Koch's postulates completed
Parrotia persica	Persian ironwood	
Photinia fraseri	Red tip photinia	
Pieris floribunda and *Pieris floribunda x japonica* & all hybrids of *P. floribunda*	Mountain Andromeda	
Pieris formosa and *P. formosa x japonica* & all hybrids of *P. formosa*	Himalaya Andromeda	
Pieris japonica & all hybrids of *P. japonica*	Japanese Pieris	
Pseudotsuga menziesii var. *menziesii* & all nursery grown *P. menziesii*	Douglas fir	
Quercus agrifolia	Coast live oak	
Quercus chrysolepis	Canyon live oak	
Quercus cerris	European turkey oak	
Quercus falcata	Southern red oak	
Quercus ilex	Holm oak	
Quercus kelloggii	California black oak	
Quercus parvula var. *shrevei* & all nursery grown *Q. parvula*	Shreve's oak	
Rhododendron spp.	Rhododendron (including azalea) – all species, hybrids and cultivars	
Rosa gymnocarpa	Wood rose	
Salix caprea	Goat willow	
Sequoia sempervirens	Coast redwood	
Syringa vulgaris	Lilac	
Taxus baccata	European yew	
Trientalis latifolia	Western starflower	
Umbellularia californica	California bay laurel, pepperwood, Oregon myrtle	
Vaccinium ovatum	Evergreen huckleberry	
Viburnum spp.	Viburnum – all species, hybrids and cultivars	

Plants Associated with *Phytophthora ramorum*

(These are regulated only as nursery stock)

Scientific Name (58)	**Common Name, Date & Source of Report**	**Notes**
Abies concolor	White fir – Oct 05 (1)	
Abies grandis	Grand fir – June 03 (1)	
Abies magnifica	Red fir – Jan 06 (7)	
Acer circinatum	Vine maple – Feb 06 (5)	
Acer davidii	Striped bark maple – Jan 06 (9)	
Acer laevigatum	Evergreen Maple – Aug 05 (3)	
Arbutus unedo	Strawberry tree – Dec 02 (7)	
Arctostaphylos columbiana	Manzanita – Feb 06 (5)	
Ardisia japonica	Ardisia – Jan 06 (9)	
Calycanthus occidentalis	Spicebush – May 05 (5)	
Castanopsis orthacantha	Castanopsis - Aug 06 (3)	New listing - Reported found in the UK
Ceanothus thyrsiflorus	Blueblossom – April 06 (5)	
Cinnamomum camphora	Camphor tree – May 06 (3)	
Clintonia andrewsiana	Andrew's clintonia bead lily – May 04 (5)	
Cornus kousa x Cornus capitata	Cornus Norman Haddon – Aug 06 (3)	New listing - Reported found in the UK
Corylus cornuta	California hazelnut – Dec 02 (5)	
Distylium myricoides	Myrtle-leafed Distylium – Jul 06 (9)	New listing - Reported found in Canada
Drimys winteri	Winter's bark – July 04 (3)	
Dryopteris arguta	California wood fern – May 04 (5)	
Eucalyptus haemastoma	Scribbly gum – Aug 06 (3)	New listing - Reported found in the UK
Euonymus kiautschovicus	Spreading euonymus – Jan 06 (9)	
Fraxinus latifolia	Oregon ash – Aug 05 (5)	
Gaultheria shallon	Salal, Oregon wintergreen – Jan 06 (9)	
Hamamelis x intermedia (H. mollis & H. japonica)	Hybrid witchhazel – Jan 06 (9)	

Hamamelis mollis	Chinese witchhazel – Jan 05 (3)	
Ilex purpurea	Oriental holly – Jul 06 (9)	New listing - Reported found in Canada
Kalmia angustifolia	Sheep laurel – May 06 (3)	
Leucothoe axillaris	Fetterbush, dog hobble – Jan 06 (9)	
Leucothoe fontanesiana	Drooping leucothoe - Oct 03 (3)	
Loropetalum chinense	Loropetalum – Jul 06 (9)	New listing - Reported found in Canada
Manglietia insignis	Red lotus tree – Aug 06 (9)	New listing - Reported found in Canada
Magnolia grandiflora	Southern magnolia – Jan 06 (9)	
Magnolia stellata	Star magnolia – Jan 05 (3)	
Magnolia x loebneri	Loebner magnolia – Jan 05 (3)	
Magnolia x soulangeana	Saucer magnolia – Jan 05 (3)	
Michelia maudiae	Michelia – Jan 06 (9)	
Michelia wilsonii	Michelia – Jan 06 (9)	
Nerium oleander	Oleander – June 06 (1)	
Nothofagus obliqua	Roble beech – Dec 04 (3)	
Osmorhiza berteroi	Sweet Cicely – Aug 05 (5)	
Osmanthus decorus (≡Phillyrea decora; ≡P. vilmoriniana)	Osmanthus – Jan 06 (9)	
Osmanthus fragrans	Sweet olive – June 06 (1)	
Osmanthus heterophyllus	Holly olive – June 06 (1)	
Parakmeria lotungensis	Eastern joy lotus tree – Jul 06 (9)	New listing - Reported found in Canada
Pittosporum undulatum	Victorian box – Dec 02 (6)	
Prunus lusitanica	Portuguese laurel cherry – Jan 06 (9)	
Pyracantha koidzumii	Formosa firethorn – Apr 04 (9)	
Quercus acuta	Japanese evergreen oak – May 06 (3)	
Quercus petraea	Sessile oak – Aug 05 (3)	
Quercus rubra	Northern red oak – Nov 03 (8)	
Rosa (specific cultivars) Royal Bonica (tagged:	Hybrid roses – Jan 06 (9)	Revised listing - Note that these are specific registered cultivars which can be identified by the

"MEImodac") Pink Meidiland (tagged: "MEIpoque") Pink Sevillana (tagged: "MEIgeroka")		listed tags
Rosa rugosa	Rugosa rose – Jan 06 (9)	
Rubus spectabilis	Salmonberry – Dec 02 (4)	
Taxus brevifolia	Pacific yew – May 03 (5)	
Taxus x media	Yew – June 05 (8)	
Torreya californica	California nutmeg – Aug 05 (5)	
Toxicodendron diversilobum	Poison oak – Dec 02 (4)	
Vancouveria planipetala	Redwood ivy – Aug05 (5)	

[1] California Department of Food and Agriculture, Sacramento, CA
[2] Oregon Department of Agriculture. Salem, OR
[3] Department for Environment, Food and Rural Affairs, UK
[4] Everett Hanson, Oregon State University, Corvallis, OR
[5] David Rizzo, University of California, Davis, CA
[6] Matteo Garbelotto, University of California, Berkeley, CA
[7] Gary Chastagner, Washington State University, Puyallup, WA
[8] Plant Protection Service, Wageningen, Netherlands
[9] Canadian Food Inspection Agency, Ottawa, Ontario, Canada
[10] (Reserved)
[11] (Reserved)

Rationale for Lists:

Host Plants Regulated for *Phytophthora ramorum:*
Naturally infected associated plants are deemed host plants regulated for *P. ramorum* upon completion, documentation, review and acceptance of traditional Koch's postulates. Details on regulated plants and articles can be found via links to "Phytophthora ramorum 7 CFR 301.92" and "Recent Modifications to Phytophthora ramorum Regulations" at: http://www.aphis.usda.gov/ppq/ispm/pramorum

The plants listed in the original Interim Rule dated 14 February 2002 were adapted from a review and evaluation of lists of regulated plants from other regulatory agencies.

Plants Associated with *Phytophthora ramorum*:
Plants associated with *P. ramorum* are naturally infected plants and from which *P. ramorum* has been cultured and/or detected using PCR (Polymerase Chain Reaction). Traditional Koch's postulates have not yet been completed nor documented and reviewed

for each of these associated plants. These reports must be documented and reviewed by PPQ before they will be listed.

Regulation at the genus level:
Plants included in either of the above lists may be regulated at the genus level. This will ensure appropriate and effective inspection in quarantine areas, regulated nurseries, and regulated articles to mitigate the spread of *P. ramorum*. An example is when the number of individual species, hybrids, or cultivars listed or to be listed is determined to hinder appropriate and effective inspection or regulation.

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APPENDIX G

INTERNET REFERENCES

http://www.aphis.usda.gov/ppq/ispm/pramorum

http://www.aphis.usda.gov/ppq/ispm/pramorum/surveyplan06/index.html

http://www.aphis.usda.gov/ppq/searchpage.html

http://www.fhm.fs.fed.us/sp/sod/sod.shtm

http://www.na.fs.fed.us/spfo/fhm/index.htm

http://www.stateforesters.org/pubs.html

http://www.suddenoakdeath.org